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Effect of Nitrogen Source on Nodulation, Nitrogen Fixation and Mineral Content of Soybean in Solution Culture

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The effect of concentration and source of added nitrogen (NH_4 and NO_3) on nodulation, nitrogen fixation, yield and content of N, P, K, Ca and Mg of soybean (*Glycine max* (L.) Merr.cv.Amsoy) were studied in nutrient solution medium. Nodulation and nitrogen fixation were severely suppressed by the addition of nitrogen source either NH_4 or NO_3. The degree of suppression was more pronounced in the presence of NO_3 than NH_4 and the reduction of nodulation and nitrogen fixation was more intensified at later stages of soybean growth. Addition of NH_4 decreased the dry weight of roots whereas NO_3 increased it and the production of tops followed the same pattern for the two forms of nitrogen studied. By the increase of NH_4 concentration in nutrient solution, N and P contents were found to increase in the roots and shoots while there was a reduction in the content of K, Ca and Mg. Unlike NH_4, NO_3 increased the uptake of cations as well.

Fixation of nitrogen by systems of root nodules of legumes is quantitatively one of the most important ways in which atmospheric nitrogen enters the biosphere. Soybean is capable of utilising the nitrogen from two sources: (1) from air (symbiotic fixation), (2) from soil (primarily nitrate). Symbiotic nitrogen fixation occurs in the root nodule bacterium, *Rhizobium Japonicum*, with soybean roots.

It has been observed that many strains of *Rhizobium* could not effect proper nodulation in many legumes such as cowpeas and vetch (Gibson and Nutman, 1950), clovers (Gibson, 1966), peas (Moustafa *et al.*, 1969) and lucerne (Munns, 1968) in the presence of combined nitrogen. Concentration of nitrogen as low as 0.5 mM may delay the nodulation when present as No_3 but has no effect when present as NH_4, asparagine or urea (Gibson, 1966). The depressive effect of combined nitrogen on nodulation and nitrogen fixation by soybean is yet to be clearly elucidated. Accordingly, the present investigation was undertaken to determine the inhibitory effect of form and dose of combined nitrogen when applied directly in the root zone. The effect of NH_4 and NO_3 on growth and mineral content of soybean was also studied.

MATERIALS AND METHODS

Seeds of soybean (*Glycine max* (L.) Merr. c.v. Amsoy) were surface

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sterilised by shaking for 20 minutes, in 3 per cent H_2O_2 containing a drop of detergent, rinsed and aerated in dilute calcium sulfate solution (Ca. 8 ppm) for 6 hours and sown in controlled environment at 26°C. After 5 days, uniform healthy seedlings were placed in plant support discs and positioned in plastic pots of 3 litre capacity which contained the nutrient solution of the following composition: Ammonium and nitrate concentrations were varied from 1 to 20 ppm added in the form of $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ $4H_2O$ respectively. The concentration of other nutrients was (in ppm) KH_2PO_4 1, KCl 20, $CaCl_2$. $2H_2O$ 10, $MgSO_4$. $7H_2O$ 2 and Fe (EDTA) Na 1. Micronutrients were supplied in the form of Hoagland A-Z solution to the amount of 1 ml/litre of nutrient solution. The nutrient solution was continuously aerated and renewed every day in order to maintain the nutrient concentrations as stable as possible. The pH of the nutrient solution was maintained at 6.5 (Lie, 1969).

Ten days after germination, the seedlings were inoculated with *Rhizobium japonicum* strain No. 315. B.3. (obtained from the Station de Phytopathologie, Gembloux, Belgium) by suspending a 5 days old culture in 50 ml of sterile water and 1ml of the resulting suspension was added to each pot (Lie., 1969). The plants were grown in a phytotron with day and night temperatures of 26 and 20°C respectively with a day period of 13 hours. Relative humidity was maintained at 75 per cent and the light intensity 16 K lux. Counting of the number of nodules was started from 5th day of inoculation till the time of harvest viz. 45 days. The experiment was replicated four times and the results are the means.

At the time of harvest, the nodules were detached carefully from the roots, rinsed with distilled water several times, dried at 105°C for 36 hours and the dry weight was recorded. Roots and shoots of the plants separated, dried and the dry weight was recorded. Total nitrogen in nodule and plant material was determined by the conventional micro Kjeldahl method. Representative samples of the plant material were digested in 10:1:4 mixture of nitric, sulphuric and perchloric acid respectively. Potassium, Ca and Mg were determined by atomic absorption spectrophotometer. Phosphorus content was analysed by molybdophosphoric blue colour method.

RESULTS

The effect of NH_4 and No_3 on the number of root nodules is shown in Table I and II respectively, at different stages of plant growth after inoculation. Pigmentation of nodules was not observed and all the nodules were uniform in colour and size, but those from control plants were bigger than the treated ones. Most of the nodules were concentrated around the tap root just below the rim of the roots and only few nodules were observed on the lateral roots. The number of nodules gradually decreased as the nitrogen concentration either NH_4 or NO_3 was increased in the nutrient solution. The control plants registered the maximum

TABLE I. Effect of NH_4 concentration on the number of root nodules in soybean. No./5 plants

NH_4 (ppm)	Days after inoculation 5	10	15	20	25	30	35
0	21	43	61	85	190	302	363
1	17	34	50	76	148	199	213
3	11	23	45	71	102	156	178
5	9	19	38	60	88	135	156
7	7	17	33	56	74	122	145
10	6	15	29	45	68	115	133
15	5	11	21	38	52	109	114
20	3	9	14	25	28	89	102
SE	0.29	0.55	0.74	0.97	2.51	3.27	3.98
LSD P=0.05	0.61	1.16	1.53	2.01	5.30	6.81	8.28
P=0.01	0.83	1.58	2.08	2.73	7.10	9.27	11.27

TABLE II. Effect of NO_3 concentration on the number of root nodules in soybean. No./5 plants

NO_3 (ppm)	Days after inoculation 5	10	15	20	25	30	35
0	21	43	61	85	190	302	363
1	14	30	36	49	79	97	115
3	9	20	32	41	53	78	89
5	7	16	25	29	39	61	75
7	7	14	23	27	32	60	66
10	5	11	20	24	31	49	55
15	3	9	16	21	26	38	43
20	2	6	8	11	18	27	30
SE	0.30	0.58	0.76	1.09	2.68	4.23	5.13
LSD P=0.05	0.62	1.22	1.59	2.28	5.58	8.79	10.67
P=0.01	0.84	1.66	2.16	3.10	7.59	11.97	14.52

number of nodules. The depressive effect of nitrogen on nodulation was more pronounced as the age of the plants advanced and the inhibitory effect has been exhibited more clearly when nitrate was present.

The amount of nitrogen fixed symbiotically at 35 days after inoculation is presented in Fig. 1. It is seen that the efficacy of nitrogen fixation decreased as the quantum of nitrogen increased in the nutrient solution. The plants without nitrogen source fixed more nitrogen than the plants supp-

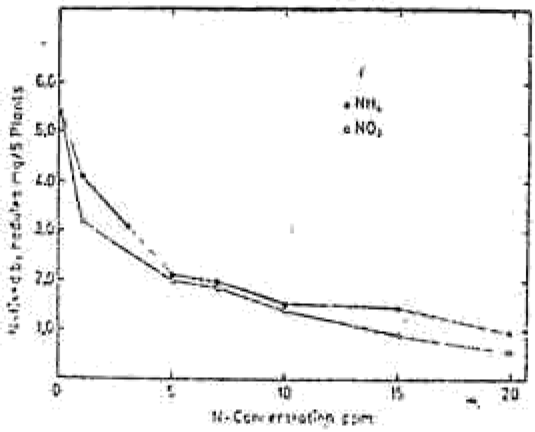

Fig. 1. Effect of NH_4 and NO_3 on the fixation of nitrogen by root nodules.

lied with nitrogen. The magnitude of nitrogen fixation was 5.36 mg/ 5 plants in the absence of nitrogen and 0.87 and 0.65 mg/ 5 plants in the presence of 20 ppm of NH_4 and NO_3 respectively. Dry weight of nodule decreased with increase in nitrogen concentration (Fig. 2).

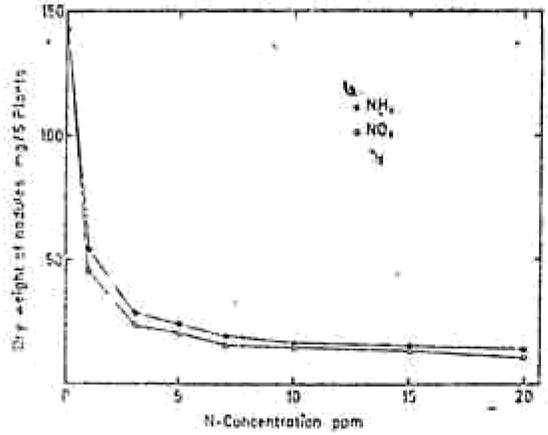

Fig. 2. Effect of NH_4 and NO_3 on the dry weight of root nodules.

It is evident from the Fig. 3 that the presence of NO_3 in the nutrient solution gradually increased the root dry weight. But the presence of NH_4

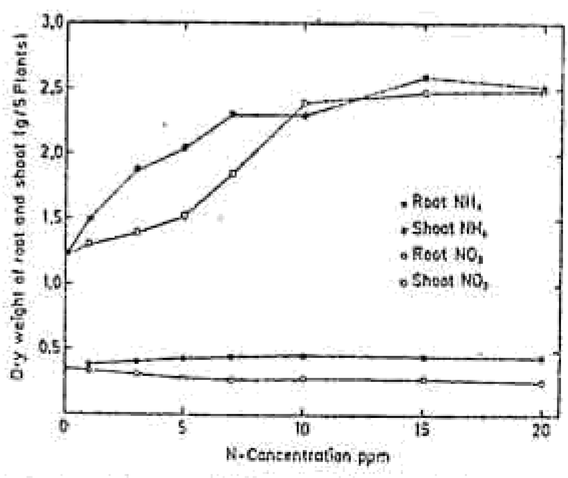

Fig. 3. Effect of NH_4 and NO_3 on the growth of soybean plants.

in the root medium depressed the root development and consequently the dry weight reduced. There was no significant difference in the shoot growth due to the different sources of nitrogen except at low concentrations. Maximum total dry matter was recorded at 10 and 15 ppm of NH_4 and NO_3 respectively.

Tables III and IV show the effect of different concentrations and sources of nitrogen on the content of N,P,K, Ca and Mg in root and shoot of soybean plants. In general, the concentrations of these elements in the plants grown under different nitrogen regimes were comparable with increased concentration of NH_4 in the root medium Nitrogen and P increased both in roots and shoots and K, Ca and Mg increased upto 5 ppm and then decreased. Though the concentrations of N and P were increased with increase in NO_3, the magnitude was not on par with NH_4. In contrast to NH_4, the presence of NO_3 in the rooting medium increased the concentration of cations in both root and shoot of soybean plants.

DISCUSSION

From the results obtained it is evident that nitrogen either as NH_4 or NO_3 suppressed the nodulation and nitrogen fixation in soybean plants grown in nutrient solution. Discontinuous production of nodules in sucessive crops has been noted previously in soybean (Bergersen, 1958). It is probably related to the more frequently described "short-term situation" where nodules appear at a rate which is initially rapid then falling sharply (Nutman, 1962; Munns, 1968) and the fall in the rate of nodule formation has

TABLE III. Effect of NH_4 on the content of nutrient elements in soybean plants (per cent on dry weight basis)

NH_4 (ppm)	N		P		K		Ca		Mg	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
0	2.78	3.22	0.19	0.25	2.08	2.13	0.30	0.54	0.19	0.47
1	3.37	3.46	0.19	0.26	1.12	2.28	0.32	0.59	0.19	0.48
3	3.56	4.18	0.20	0.27	2.29	2.48	0.37	0.60	0.20	0.49
5	3.94	4.28	0.21	0.28	2.28	2.52	0.39	0.61	0.21	0.49
7	4.10	4.43	0.21	0.29	2.28	2.48	0.41	0.59	0.20	0.49
10	4.23	4.69	0.21	0.29	2.15	2.40	0.38	0.58	0.18	0.45
15	4.38	4.97	0.22	0.31	1.13	2.33	0.36	0.56	0.16	0.45
20	4.76	5.19	0.23	0.32	1.07	2.20	0.32	0.53	0.12	0.43
SE	0.03	0.04	0.01	0.001	0.04	0.01	0.002	0.001	0.001	0.001
LSD P=0.05	0.06	0.08	0.01	0.003	0.07	0.01	0.004	0.002	0.003	0.002
P=0.01	0.08	0.11	0.02	0.004	0.09	0.04	0.01	0.003	0.004	0.003

TABLE IV. Effect of NO_3 on the content of nutrient elements in soybean plants (per cent on dry weight basis)

NO_3 (ppm)	N		P		K		Ca		Mg	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
0	2.78	3.22	0.19	0.25	2.08	2.13	0.30	0.54	0.19	0.47
1	2.53	3.29	0.09	0.22	3.08	2.40	0.27	0.49	0.09	0.45
3	2.66	3.29	0.10	0.23	3.58	2.56	0.28	0.51	0.09	0.44
5	2.86	3.39	0.10	0.25	3.89	2.79	0.29	0.52	0.09	0.44
7	2.97	3.52	0.10	0.25	4.19	2.84	0.30	0.54	0.09	0.43
10	3.08	3.74	0.11	0.26	4.24	3.01	0.32	0.57	0.09	0.47
15	3.20	3.90	0.11	0.26	4.32	3.22	0.32	0.59	0.10	0.48
20	3.24	3.95	0.12	0.28	4.41	3.31	0.33	0.59	0.10	0.48
SE	0.026	0.047	0.001	0.006	0.038	0.018	0.001	0.002	0.002	0.001
LSD P=0.05	0.055	0.098	0.003	0.012	0.080	0.037	0.002	0.004	0.003	0.002
P=0.01	0.075	0.133	0.004	0.016	0.108	0.050	0.002	0.005	0.004	0.002

been attributed to the first established nodules restraining further nodulation. In the present investigation also the formation of nodules become less at later stages of plant growth.

Large amounts of combined nitrogen, in excess of those likely to be present naturally in soils, are expected to suppress the nodule development. Bjorkman (1942) grew alders in a humus-sand mixture and observed that in the presence of 100mg of ammonium nitrate per litre of the culture solution, the volume of nodules formed was reduced from 33 cu. mm per plant (the value with no added nitrogen) to 15 cu. mm. Nodulation was almost completely suppressed in the presence of 200 mg nitrogen per litre and above. Quispel (1954) reported that after 7 weeks growth, a level of approximately 5 mg of NH_4-N per litre was found to have reduced the dry weight of nodules per plant to half while

50 mg completely suppressed nodulation in Alnus plants. It is interesting to note that addition of 5 mg of NH_4 of NO_3 caused about 50% reduction in the number and dry weight of nodules when compared to the treatments without nitrogen source. The results observed in this study are in good agreement with the findings of Moustafa *et al.* (1969) and Hoglund (1973).

It could be seen from Fig. 3 that NH_4 decreased the dry weight of root but NO_3 on the contrary increased it. Cox and Reisenauer (1973) reported that NH_4 at a concentration of 80-100 μM produced symptoms of incipient NH_4 toxicity in wheat. In the present investigation, only the root development was affected by the addition of NH_3 and this may be due to the effect of NH_4 on respiration (Gibson 1966) or due to the interaction of NH_4 and K in their role in the physiology of the roots (Barker *et al.* 1966). Our previous study (Joseph *et al.*, 1976) has also revealed the depressive effect of NH_4 on root development in soybean plants. Most of the plants tolerate a large excess of NO_3 and accumulate it within their tissues (Cox and Reisenauer, 1973) and this may be the reason why the root development of the plants supplied with NO_3 was not affected in the present study.

The effect of NH_4 on N and P content is comparable with the results of Cox and Reisenauer (1973), where the content of N, P and S increased both in tops and roots of wheat as the NH_4 concentration increased in the nutrient solution and at the same time K, Ca and Mg content decreased. Bouma (1970) suggested that the decline in P content during nitrogen stress was at least partly related to a decline in chlorophyll content. The decrease of cations with the increase in NH_4 in the nutrient solution is also comparable with the results of Cox and Reisenauer (1973). With the increase in NO_3, cation concentration increased in both shoots and roots. The NH_4 induced increase in intake of anions, with concomitant reduction in cation adsorption, is in agreement with the findings of Harada *et al.*, (1968) and Joseph (1976). This effect is generally considered to be the result of stimulated anion intake in response to an overall increase in cations (largely NH_4) intake. The total N content in the plant parts was greater when NH_4 is added rather than NO_3. Similar results have also been reported by Bouma (1970).

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